confirmed to be the fragments of interest by analyzing their base sequences. The amplified fragments were recovered by ethanol precipitation and dissolved in 10 mM carbonate buffer (pH 9.5) at a concentration of 1 μM. In addition, β-actin gene as a housekeeping gene and a plasmid pUC18 as a negative control were similarly prepared. These fragments were spotted onto a slide glass with introduced amino groups (Sigma) using an instrument for preparing DNA chips (GMS) and fixed by UV irradiation. The slide glass was washed with 0.2% SDS followed by distilled water and dried to prepare a DNA array.

Example 2

(1) Administration into mouse

10 female mice (2 days old) were divided into a group with endocrine disruptor administration and a group without administration. Diethylstilbestrol (DES), which potentially causes endocrine disruption, was intravenously injected into each mouse in the group with administration at 0.1 mg/mouse/day for 2 days. Ovaries were removed on day 4, and mRNAs were prepared using an mRNA extraction kit (Qiagen).

cDNAs synthesis reactions were carried out using mixtures each containing about 3 μg of the mRNA, oligo-dT primer, Cy3-dUTP (Amersham) for the group with administration or Cy5-dUTP (Amersham) for the group without

administration, dNTPs and reverse transcriptase (Gibco-BRL). The mixtures were subjected to gel filtration, concentrated under reduced pressure and dissolved in 4 x SSC/0.2% SDS to prepare fluorescence-labeled cDNAs.

(2) Treatment of cultured cells

Human breast cancer MCF-7 cells were grown in DME medium containing 5% fetal bovine serum (FBS). After trypsinization, 2 x 10^5 cells were placed in each well of a 12-well culture plate. The cells were incubated in the same medium for 24 hours. After the medium was removed, the cells were cultured for 72 hours in DME medium containing 5% human serum from which steroid hormones had been removed by treatment with activated carbon-dextran in the presence or absence of 17-β estradiol at a concentration of 10 pM. The cells were recovered, and mRNAs were extracted as described in Example 2-(1).

cDNAs synthesis reactions were carried out using mixtures each containing about 3 μg of the mRNA, oligo-dT primer, Cy3-dUTP for the cells exposed to 17-β estradiol or Cy5-dUTP for the cells not exposed to 17-β estradiol, dNTPs and reverse transcriptase (Gibco-BRL). The mixtures were subjected to gel filtration, concentrated under reduced pressure and dissolved in 4 x SSC/0.2% SDS to prepare fluorescence-labeled cDNAs.

(3) Hybridization of labeled cDNA with DNA array

Equal volumes of the Cy3-labeled cDNA and the Cy5-labeled cDNA as prepared in (1) above were mixed together and heat-denatured. 5 µl of the mixture was dropped onto the DNA array as prepared in Example 1. A cover glass was placed on the mixture and the sides of the cover glass were sealed with a film. After incubation at 40-45°C for 10 hours, the cover glass was removed. The DNA array was washed in 0.2 x SSC/0.1% SDS for 30 minutes and in 0.2 x SSC for 30 minutes, and then air-dried. The fluorescent signals from the respective spots on the DNA array were analyzed using a microarray scanner (GMS). Furthermore, similar procedure was carried out for the labeled cDNAs obtained in (2) above.

As a result, significant changes in signals were observed for the ovary from the mouse administered with DES and MCF-7 cells exposed to 17-β estradiol. Thus, genes that were influenced by endocrine disruptors could be detected.

Example 3

(1) Preparation of DNA array

33 genes were selected from the genes listed in Table 3 in Example 1. The selected genes are shown in Table 4. β-Actin gene and a plasmid pBR322 were used as a housekeeping gene and as a negative control, respectively.